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Effects of PCBs sorbed to algal paste and sediments on the stress protein response (HSP70 family) in the eastern oyster, *Crassostrea virginica*

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Abstract

This study examined the stress protein response (HSP70 family) of reproductively inactive oysters fed 0.7 g algal paste containing 0, 0.35 and 3.5 μg polychlorinated biphenyls (PCBs) daily. A second set of treatment groups investigated the combined effect of PCBs and sediments (0.3 g sediments daily per oyster) on HSP70 response. After 8 weeks of PCB exposure, oyster tissues (mantle and gill) were sampled and analyzed for HSP70. Preliminary results did not show a significant effect in HSP70 response in oysters fed PCB sorbed to algal paste, albeit PCBs accumulated up to 1342 ng/g dry weight in the mantle, and up to 180 ng/g dry weight in gill tissues. However, the addition of sediments caused a significant increase in HSP70 levels of gills and mantle, although the mantle was less sensitive to the sediments.

Keywords: Polychlorinated biphenyls; Stress proteins; Biomarkers; Oysters; *Crassostrea virginica*

In the Chesapeake Bay region, declines in populations of the eastern oyster *Crassostrea virginica* have been attributed to disease and a decrease in water quality due to pollution. Commercial polychlorinated biphenyls (PCBs) are of particular concern due to their high chemical stability, hydrophobicity and low degradation in the

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environment. PCBs once in the food web can bioconcentrate in successively higher trophic levels.

Stress proteins (SPs) are known to be synthesized at elevated levels when organisms are challenged with environmental stimuli such as high temperature, and toxic chemicals making them a potentially useful marker of exposure (Morimoto, 1993). Members of the HSP70 comprise one of the most highly conserved proteins known, and has shown good induction and conservation of reaction across species, including marine invertebrates such as mussels (*Mytilus edulis*), Pacific oyster (*Crassostrea gigas*), and echinoderms (*Strongylocentrotus purpuratus*) (Clegg, Uhlinger, Jackson, Cherr, Rifkin & Friedman, 1998; Sanders, 1990).

The present study was conducted to determine whether a SP response by members of the HSP70 family is elicited in oysters via a PCB-containing diet. The combined effect of PCBs and sediments on the HSP70 response was investigated by simultaneous exposure to 0.3 g clean artificial sediments (Illite 46E0315, Wards/Cenco, Rochester, New York).

Oysters from Maine, an area rarely infected by the parasite *Perkinsus marinus,* were placed in individual 2-l containers with aeration and fed daily 0.7 g of algal paste containing 0, 0.35, or 3.5 μg PCBs. Water in the containers was changed every other day. PCB-sorbed algal paste was prepared by mixing PCBs (1:1:1 mixture of Aroclor 1242, 1254, and 1260) dissolved in acetone with algal paste. PCBs were extracted from the filtered paste and $>99.8\%$ was found sorbed to the algae. To study the combined effects of sediments and PCBs on HSP70 expression, 0.3 μg sediments daily/oyster were added to a second set of containers with the above-mentioned treatments. Little work has investigated the impact of exposure to suspended sediments on PCB assimilation from contaminated algae. This may be critical due to the high-suspended sediment loads of many coastal and estuarine waters.

After 8 weeks of exposure oysters spawned ($n=29$) and tissues (mantle and gill) were sampled and analyzed ($n=15$/treatment) for SPs (HSP70 family). SPs were determined using Slot Blot (Bio Dot SF microfiltration) and detected by primary monoclonal antibody (MA3-006 Affinity BioReagents) followed by the application of Goat anti-mouse antibody conjugated to alkaline phosphatase. The primary monoclonal antibody (MA3-006) recognized isoforms of 69 and 71 KDa in the eastern oyster gill and mantle (unpublished results), and has been shown to react in HeLa cells with HSP70, HSC70, p75, and HSP72 (manufacturer information). The recognized members are thus referred to as HSP70 family.

Bands developed were quantified using a densitometer (Enprotech scanner and software). Data were log-transformed and analyzed using two-way analysis of variance (ANOVA) for effects of PCB dose and sediment (SAS Institute, Cary, NC, USA). Total PCBs in oyster were extracted from tissues by supercritical fluid extraction and analyzed by gas chromatography according to Hale and Gaylor (1995). A condition index was calculated as tissue dry weight divided by dry shell weight.

Our results show no significant effects ($P<0.05$) in mean total HSP70 response in oysters due to PCB-only exposure (Fig. 1) in either of the tissues analyzed, although PCBs accumulated up to 1342 ng/g dry weight in the mantle and up to 180 ng/g dry weight in gill tissues (Fig. 2). The presence and the amount of denatured proteins in

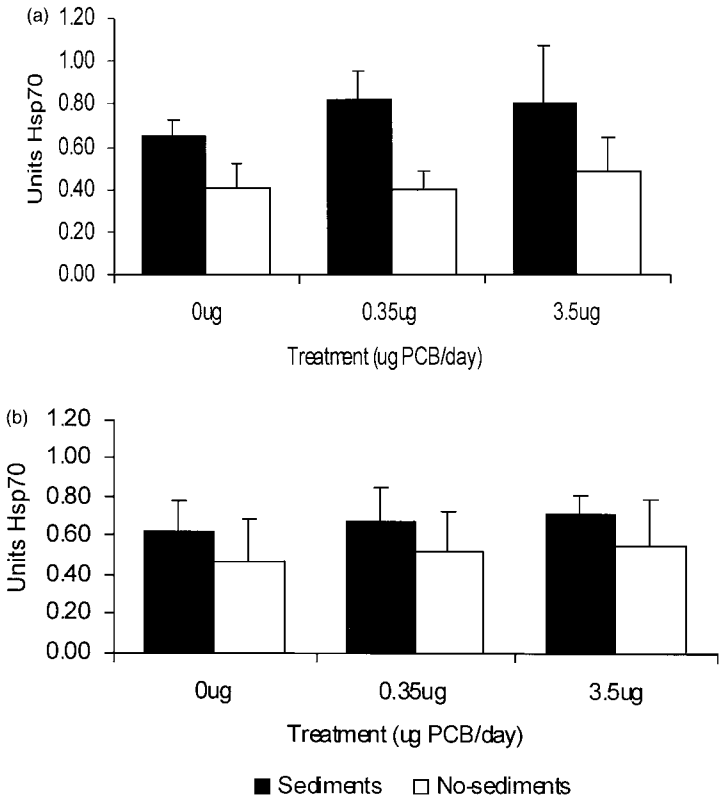

Fig. 1. (A) Total Hsp70 response in gill of oysters exposed to PCB sorbed to algal paste and artificial sediments (mean ±95% C.I., $n=9$–10). (B) Total Hsp70 response in mantle of oysters exposed to PCB sorbed to algal paste and artificial sediments (mean ±95% C.I., $n=8$–9). No dose effects due to PCB exposure were observed in the tissues analyzed. The addition of sediment produced a significant increase in total mean HSP70 response in both tissues.

the cell have been considered requirements for eliciting a SP response (Baler, Welch & Voellmy, 1992). Our results suggest that neither PCB concentration used in the present study, nor the length of exposure produced sufficient protein denaturation in the oyster to elicit a stress response above constitutive levels. In addition, no significant differences were observed in the condition index between PCB-exposed and non-PCB-exposed, and between sediments and non-sediments-exposed oysters.

The addition of sediment caused a significant increase in gill and mantle HSP70 levels ($P<0.05$) compared to the non-sediment treatments (Fig. 1). However, gills appeared to be more sensitive to the sediment load than mantle. The presence of inorganic particulates may cause mechanical damage to the gill by scouring the surfaces of tissues, eliciting the HSP70 response observed. In mantle the smaller increase between sediment and non-sediment groups, compared to that exhibited in the gills (Fig. 1), may be indicative of a systemic stress response.

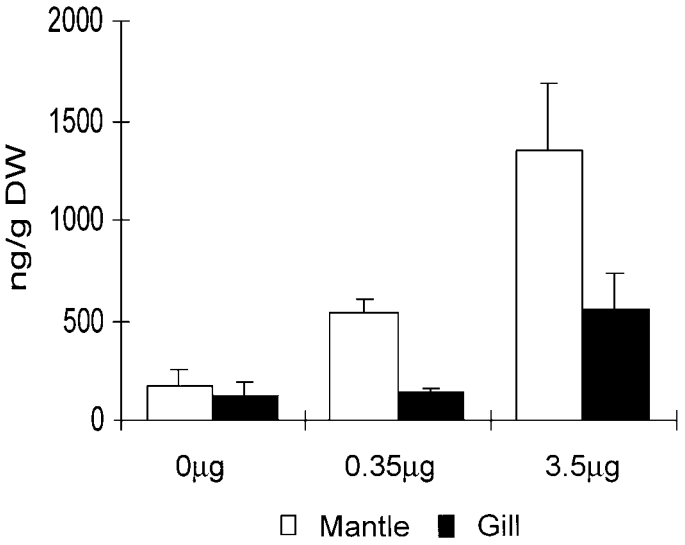

Fig. 2. Total PCB accumulation in gills and mantle versus daily PCB dose administered via a contaminated algal diet, no sediments were added (mean ±S.D.).

Sediment loads have been linked to adverse effects on the gills of aquatic organisms including bivalves (Stevens, 1987; Whitfield & Paterson, 1995). Clogging of the gills by sediments is the primary cause of mass fish mortality associated to floods in South African estuaries. In *Pecten novaezelandiae,* excised gill tissues showed less tolerance to fine silt/clay ($\leqslant 10$ µm) sediments (Stevens, 1987).

PCBs have been implicated as causative agents of lipid peroxidation in rats through the formation of free radicals (Kamohara, Yagi & Itokawa, 1984) with the potential to cause damage to cell membranes and produce protein denaturation. In marine invertebrates PCBs may have less severe consequences. Metabolic processes (biotransformation enzymes) are slower in marine invertebrates, resulting in a reduced formation of potentially more harmful metabolites. It has been suggested that levels of free-radical scavengers, such as carotenoids, are particularly high in marine invertebrates and counter more immediate effects of free radicals (Livingstone, 1992). Alternatively, the absence of deleterious effects by PCBs observed in marine invertebrates could be due to low binding affinity between the aryl hydrocarbon hydroxylase (AHH) receptor and PCBs (Denison, Wilkinson & Okey, 1986).

PCBs preferentially bioaccumulate in lipid-rich tissues (Chu et al. 1999, abstract) of oysters. Stored in these lipids, hydrophobic contaminants may be precluded from exerting toxic effects on the organisms. The addition of sediments produced an increase in the stress response by members of the HSP70 family. Exposure of the gill surface to particulates may result in immediate damage, eliciting an additional HSP70 response. HSP70 response in mantle may be a result of a systemic response by the organisms to this stressor.

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